ORIGINAL ARTICLE

Comparison of Antinociceptive activity of Fluoxetine with Aspirin in Albino Rats

A Uma [1], Sarasija V [2], Gattu P Goud [3]

Tutor [1], Assistant Professor[2], Dept of Pharmacology, RIMS Adilabad, Telangana, India
[3]Tutor, Dept of Pharmacology, Siddhpur Dental College & Hospital, Patan, Gujarat, India
http://dx.doi.org/10.18049/jcmad/319a14

Abstract

***Background**: Pain is a complex unpleasant sensation and a very common phenomenon that has sensory-discriminative, cognitive-evaluative, and affective-emotional dimensions. The principal objective of the treatment of pain is to remove or abolish the cause of pain. But it is not always possible to do so. Hence, analgesics are used for the symptomatic treatment of pain. Finding newer and potent analgesics is an area of interest for many researchers with this background we in the present study tried to compare antinoceptive activity of Fluoxetine and Aspirin in Animal model (Albino Rats). **Methods**: Wistar albino rats (150-200 gms), bred in the Central Animal House National Institute of Nutrition, Hyderabad were used. The animals housed under standard laboratory conditions. Model for pain was Chemical induced pain using acetic acid in albino rats. The animals were acclimatized to laboratory conditions fifteen days before the test. Each animal was used only once in the experiment. Fluoxetine hydrochloride pure powder by Sigma Aldrich pharmaceuticals and Aspirin 3% was obtained from Dept of Pharmacy Lab KIMS Hyderabad were used during the study. **Result**: Pretreatment with Fluoxetine (5mg/kg,i.p.) 30 min. before the injection of acetic acid, delayed the onset(12.00±1.67 min.), decreased the number of wriths (8.17±0.75) and total duration of writhing (17.17±1.47 min.) which are statistically significant in comparison to normal saline pretreated rats(onset 3.67±1.03 min, number of wriths 11.67±1.86, total duration of writhing 23.33±1.97 min.. Pretreatment with Fluoxetine (10mg/kg,i.p.) 30 min. before the injection of acetic acid, also delayed the onset(16.00±0.98 min.), decreased the number of wriths (6.83±0.75) and total duration of writhing (15.15±1.05 min.) which are stastically significant in comparison to normal saline pretreated rats(onset 3.67±1.03 min, number of wriths 11.67±1.86, total duration of writhing 23.33±1.97 min).Pretreatment with standard drug Aspirin (100mg/kg,i.p.) 30 min. before the injection of acetic acid, delayed the onset(16.33.0±1.03min.), decreased the number of wriths (6.33±0.82) and total duration of writhing (14.17±0.75 min.) which are stastically significant in comparison to normal saline pretreated rats onset 3.67±1.03 min, number of wriths 11.67±1.86, total duration of writhing 23.33±1.97 min. **Conclusion**: The present study shows that Fluoxetine has antinociceptive effect in experimental animal models of pain by chemically induced Writhing test in albino rats. Fluoxetine has produced similar antinociceptive effect at doses of 10 mg/ kg when compared to Aspirin (100mg/Kg) in albino rats. Indicating Fluoxetine is more potent antinociceptive drug than Aspirin in the acetic acid induced writhing model in albino rats.*

***Keywords:** Antinociception, Fluoxetine and Aspirin*

Address for correspondence: A Uma, Tutor, Dept of Pharmacology, RIMS Adilabad, Telangana, India. Mob: 9908645456,8885068813.Email:umareddyallala@gmail.co,dr.sarasija@gmail.com,prakashgoud005@gmail.com

Introduction

Pain is an ill defined, unpleasant sensation usually evoked by an external or internal noxious stimulus. The taxonomy committee of International Association for the study of pain defines pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [1]. Pain being a subjective phenomenon is perceived only by the sufferer [1]. Pain acts as a warning signal against disturbances either in the body or in the external

environment of an individual. From the time of vedic civilization, there have been attempts to find pain relief with healing touch and the extracts from herbs and bushes. In the early part of post-christ era, Galen strongly proposed the concept of pain relief from plant extracts and various other methods [2]. Although principal objective of treatment of pain is to remove cause of pain but may not be possible in all conditions. Hence, analgesics are used for the symptomatic treatment of pain. Non - Steroidal Anti Inflammatory Agents [NSAIDs] drugs are among the most widely used of all therapeutic agents, frequently prescribed for musculoskeletal pain but often taken without prescription for miner aches and pains. They act primarily on peripheral pain mechanisms, also in CNS to raise pain threshold. Aspirin is the prototype of these drugs which includes a variety of different chemical classes [3].

Mechanism of action of NSAIDs: [4] All these effects are related to the primary action of the drugs – inhibition of arachidonic acid cyclooxygenase and thus inhibition of production of prostaglandins and thromboxanes – through some aspects of the action of individual drugs may occur by different mechanisms. These two isozymes of cyclooxygenases, COX-1 and COX-2. COX-1 is an enzyme present in all tissues including blood platelets and is involved in cell to cell signaling and in tissue homeostasis. COX-2 is induced in inflammatory cells when they are activated and produces prostanoid mediators of inflammation. NSAIDs vary in their degree of inhibition of both isoenzymes thus in their ability to produce unwanted defects (COX – 1 inhibition) and anti inflammatory effects (COX-2 inhibition). Aspirin causes irreversible inactivation of these enzymes, It acetylates the serine 350 that is at the apex of the long hydrophobic channel in the main cyclooxygenase -1 site and there by excludes archidonates from the channel.

Prostaglandin sensitizes the nociceptive afferent nerve terminal to mediators such as bradykinin and serotonin. NSAIDs are effective in those types of pain where inflammation and tissue damage causes increase in synthesis of prostaglandins. Eg: arthritis, bursitis, myositis, vasculitis, dysmenoorrhoea, cancer metastasis. In combination with opioids they reduce the requirement of opiods to almost one-third. Their ability to relieve headache may be related to the decrease of vasodilator effect of prostaglandins on cerebral vasculature. Recent evidence shows that NSAIDs when injected to spinal canal produce analgesia, suggesting that inhibition of prostaglandin generation within the spinal cord may contribute to their analgesic action.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) belonging to antidepressants. Selectively inhibit reuptake of serotonin and thereby increase synaptic serotonin levels, also cause increased 5HT receptor activation and enhanced postsynaptic responses. At high doses, also bind NE transporter [5]. SSRIs inhibit the reuptake of the serotonin (5-HT), increase the level of 5-HT in the neuronal synapse, and facilitate serotonergic activity. This mechanism may be involved in the antinociceptive effect of antidepressants [6]. There are several areas of the CNS that, directly or indirectly, are activated by nociceptive inputs, are targets of opioids, and participate in the central modulation of pain [7-12]. They include the prefrontal, anterior cingulate, and insular cortices, amygdale, periventricular and posterolateral hypothalamus,PAG, dorsolateral pons, and RVMM. These brain areas exert bidirectional influences on pain sensation as they may either inhibit or facilitate transmission of nociceptive inputs at the level of the dorsal horn [8, 9]. These modulatory effects are largely mediated by descending monoaminergic pathways that utilize serotonin, norepinephrine, or dopamine [12]. The bioavailability of fluoxetine is relatively high 72%. Peak plasma concentrations are reached in 6 to 8 hours. Fluoxetine is extensively metabolized in the liver to norfluoxetine and a number of other unidentified metabolites. The only identified active metabolite, norfluoxetine, is formed by demethylation of fluoxetine. It is used to treat major depression, bipolar disorder, obsessive compulsive disorder, bulimia nervosa, panic disorders and premenstrual dysphoric disorder [8]. It is also used for the treatment of cataplexy, obesity, alcohol dependence as well as binge eating disorder [8, 9].

With this prospective, in this study an attempt has been made to assess the antinociceptive activity of fluoxetine, a selective serotonin reuptake inhibitor compared with Aspirin in animal models.

Materials and Methods

The present study is undertaken to study antinociceptive potential of Fluoxetine in animal models of pain in albino rats. It was conducted in the Department of Pharmacology, Kamineni Institute of Medical Sciences. The study was placebo controlled randomised laboratory based comparative study on animals with prior permission of institutional Animal Ethics Committee and was executed according to the guidelines of the committee for the purpose of control and supervision of the experiments on animals (CPCSEA), India. Wistar albino rats (150-200grms), bred in the Central Animal House National Institute of Nutrition, Hyderabad were used. The animals were acclimatized to laboratory conditions fifteen days before the test. Each animal was used only once in the experiment. The animals housed under standard laboratory conditions. Fluoxetine hydrochloride pure powder – Sigma Aldrich pharmaceuticals Aspirin, Acetic acid 3%, Department of Pharmacy Lab, KIMS. Model of pain was by Chemical induced pain using acetic acid in albino rats.

Chemical Induced Writhing Test

Rats of either sex were selected randomly numbered, weighed and divided in to five groups, each having 6 rats. Normal saline, fluoxetine, aspirin were injected 30minutes before i.p. injection of 3%v/v acetic acid diluted in distilled water, at a dose of 10ml / kg. The three parameters – onset, number, duration of writhes are counted upto 30min after acetic acid injection. (writh is characterized by contraction of abdominal muscle followed by extension of hind limbs). Reduction in number of wriths, delay in onset and decreased duration of wriths compared to placebo group was considered as presence of antinociception.

Table- 1: Grouping of rats (n = 6)

Groups	Drugs	Dose mg/kg
I (Control)	Normal saline	1 ml
II (Standard)	Aspirin	100
III (Test drug)	Fluoxetine	2
IV (Test drug)	Fluoxetine	5
V (Test drug)	Fluoxetine	10

Table 1 showing the 30 rats divided into five groups (Group I to Group V) n=6 with drugs and the dosage used.

Figure- 1: Writhing phenomenon

Results

Writhing was produced by administering 3% acetic acid (10 ml/kg, i.p.) in albino rats & onset, number of wriths, duration of total writhing period in the observation period of 30 min. was noted. Table 2 shows the comparison of writhing test in placebo group who were pre-administered Normal Saline (NS) 1ml/Kg with Aspirin group administered 100mg/Kg. the onset of wriths Number of wriths and duration of each was recorded separately. The Average onset was delayed in the Aspirin group and the total number of wriths was reduced and duration of each writh was also reduced.

Figure 2: Comparison of Number of Stretches of Writing test in all groups.

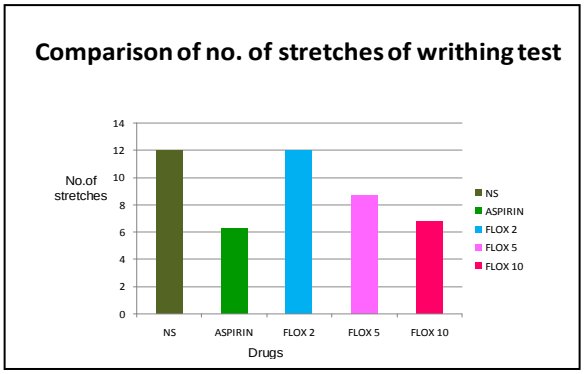

Table 3 shows writhing test performed in three groups Group III, IV, V who were pre-treated with Fluoxitine 2mg/kg, 5mg/Kg and 10mg/Kg respectively the onset of each writh, Number of wriths in 30 minute duration and total duration of each writh were recorded and compared as seen from the table as the fluoxitine dosage increases the onset gradually increases and the number of wriths and duration of writh gradually decreases.

Table 2: Writhing test- placebo group (NS) 1ml/kg and Aspirin (100mg/kg) group

Weight (gms)	Dose (mg/ml)	Placebo group (NS) 1ml/kg Writhing (min.)			Aspirin (100mg/kg) Writhing (min.)				
		Onset	No. of Wriths	Duration	Weight (gms)	Dose (mg/ml)	Onset	No. of Wriths	Duration
180	0.18	3	12	20	190	0.8	15	7	15
160	0.16	3	15	25	160	0.6	15	7	14
185	0.18	3	10	24	190	0.8	18	6	15
175	0.17	3	11	25	180	0.7	18	6	14
180	0.18	5	10	24	175	0.7	15	7	14
165	0.16	5	12	22	165	0.6	17	5	13

Table 3: Writhing Test fluoxitine 2mg/Kg, 5mg/Kg, 10mg/Kg (*= Duration)

Dose (mg/ml)	Fluoxitine 2mg/Kg			Dose (mg/ml)	Fluoxitine 5mg/Kg			Dose (mg/ml)	Fluoxitine 10mg/Kg		
	Onset	No. of Wriths	D*		Onset	No. of Wriths	D*		Onset	No. of Wriths	D*
0.31	3	13	21	0.8	12	9	20	0.82	15	8	15
0.29	5	15	22	0.9	10	9	17	0.85	17	6	17
0.36	5	10	22	0.8	12	8	17	0.82	16	7	16
0.39	4	12	24	0.9	15	7	16	0.9	17	6	14
0.31	5	11	22	0.87	12	8	16	0.85	15	7	16
0.35	6	11	21	0.8	11	8	17	0.9	17	7	15

Table 4: Shows the Average values calculated with Standard deviation values for each group

Group (n=6)	Dose (mg/kg)	Wriths Mean±SD		
		Onset (min)	Number	Duration (min)
Control	1 ml/kg	3.67±1.03	11.67±1.86	23.33±1.97
Aspirin	100	16.33±1.51	6.33±0.82	14.17±0.75
Fluoxetine	2	4.67±1.03	12.00±1.79	22.00±1.10
Fluoxetine	5	12.00±1.67	8.17±0.75	17.17±1.47
Fluoxetine	10	16.17±0.98	6.83±0.75	15.50±1.05

Table 5: Inter - group comparison of Writhing test by multiple comparison test (LSD test)

Groups	Onset	Number	Duration
NS vs. A	12.66**	5.33**	9.16**
NS vs.F2	1.00^{ns}	0.33^{ns}	1.33^{ns}
NS vs.F5	8.33**	3.55**	6.16**
NS vs.F10	12.5**	4.83**	7.83**
A vs.F2	11.66**	5.66**	7.83**
A vs.F5	4.33**	1.83*	3.00**
A vs.F10	0.16^{ns}	0.50^{ns}	1.33^{ns}
F2 vs.F5	7.33**	3.83**	4.83**
F2 vs.F10	11.55**	5.16**	6.50**
F5 vs.F10	4.16**	3.83^{ns}	1.66*

NS – Normal saline, **A**-Asprin , **F** - Fluoxetine **ns** – not significant, * P < 0.05, ** P < 0.001

The control group had average onset of duration 3.67±1.03 (min) the number of wriths 11.67±1.86 (min) and duration of each writh lasted for 23.33±1.97 (min). Prior treatment with Aspirin 100mg/Kg delayed the onset of wriths to 16.33±1.51 (min) the number of wriths were 6.33±0.82 (min) and duration of writh was 14.17±0.75 (min) respectively. Pretreatment with Fluoxetine 2mg/Kg onset of duration 4.67±1.03 (min) the number of wriths

12.00±1.79 (min) and duration of each writh lasted for 22.00±1.10 (min). Pretreatment with Fluoxetine (5mg/kg,i.p.) 30 min. before the injection of acetic acid, delayed the onset(12.00±1.67 min.), decreased the number of wriths (8.17±0.75 min) and total duration of writhing (17.17±1.47 min.) Fluoxitine 10mg/Kg had average onset of duration 16.17±0.98 number of wriths were 6.83±0.75 and duration of wriths were 15.50±1.05 (min) table- 4.

Comparison of mean values of three groups the Control group with Normal Saline dosage of 1mg/kg (NS) and the two test groups one of Aspirin with dosage of 100mg/kg (A) and Fluoxetine with three different dosages of 2mg/Kg (F2), 5mg/kg (F5)and 10mg/Kg (F10) respectively table 5. One way Anova analysis was done and Fischer's Least Significant Difference (LSD) was performed. Comparison of NS with A group shows all the three values of onset the number and duration were significant however when NS is compared with F2 group the values are not significant for any parameter. NS when compared with F5 and F10 respectively also had significant values for onset number and duration of wriths.

Comparison of A (Aspirin) with F2 and F5 had all the parameters significant but Aspirin (A) when compared with F10 had all the values Not significant. An inter group comparison of F2 Vs F5 and F2 Vs F10 shows significant values for all parameters similarly F5 Vs F10 shows onset and duration as significant but the Number of wriths were not significant.

Discussion

Non-steroidal anti-inflammatory drugs are mainly used for treatment of integumental pain, and act mainly by peripheral mechanism of action by inhibiting prostaglandin synthesis. These drugs are more effective against pain associated with inflammation and adverse effects like CNS depression and dependence are less when compared to opioids. Since they are less effective for treatment of pain disorders associated with depression there is always search for other modalities of treatment needed for treating this pain associated with depression disorders. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) belonging to antidepressants. The neurotransmitter serotonin (5-hydroxytryptamine or 5-HT), which is the target for many of the drugs used to treat depression. Many of these antidepressant medications also affect norepinephrine (NE) neurotransmission. Both neurotransmitter pathways are believed to be central to the modulation of mood. Though many of the drugs presented function as antidepressants and hypnotics, other medications in this pharmacologic group are effective treatments for migraine, headache and irritable bowel syndrome. The effects of descending monoaminergic systems on nociceptive processing in the dorsal horn are complex. Monoamines may act via different subtypes of receptors located at the primary nociceptive afferents, dorsal horn projection neurons, local excitatory or inhibitory interneurons, and glial cells [10, 13]. Serotonin, norepinephrine, and dopamine may exert either antinociceptive or pronociceptive effects according to the type of receptor involved, site of action in the dorsal horn, and crosstalk between descending and local neurochemical signals, including adenosine, endogenous opioids, and nitric oxide [10] . In addition, presynaptic reuptake via selective transporters and control of release via presynaptic inhibitory autoreceptors regulate the local concentration of monoamines and thus their effects on the targets in the dorsal horn [10]. These monoaminergic systems have an important role in mechanisms of inflammatory and neuropathic pain [9] and are a target for pharmacologic management of these conditions. Recently, numerous open and controlled studies have shown that antidepressant drugs also have analgesic activity and particularly, selective serotonin reuptake inhibitors (SSRI) like Fluoxetine are effective in mixed, chronic pain [14]. It has also been shown that some antidepressants are superior to placebo in about 75% of studies [15]. A wide range of pain conditions are responsive to antidepressants, particularly diabetic neuropathy pain and probably rheumatoid arthritis [16] and migraine [17], fibromyalgia [14]. SSRIs inhibit the reuptake of the serotonin (5-HT), increase the level of 5-HT in the neuronal synapse, and facilitate serotonergic activity. This mechanism may be involved in the antinociceptive effect of antidepressants [6].

In the present study we compared the analgesic effect of aspirin a standard drug belonging to

class of NSAIDs with antidepressant Fluoxitine in animal model with chemically induced pain method. Although flouxitine in doses of 2mg/Kg and 5mg/Kg produced nociceptive actions but fluoxitine 10mg/Kg produced nociceptive effects comparable to Aspirin 100mg/Kg. Singh *et al* 2001[17] also reported antinociceptive effect of fluoxetine in albino mice like our present study in the writhing test model. Pretreatment with standard drug Aspirin (100mg/kg,i.p.) 30 min. before the injection of acetic acid, delayed the onset(16.33.0±1.03min.), decreased the number of wriths(6.33±0.82) and total duration of writhing (14.17±0.75 min.) which are stastically significant in comparison to placebo group. Antinociceptive effect of Fluoxetine 10 mg/kg i.p. was not statistically significant in comparison to Aspirin 100 mg/kg i.p. Suggesting that Fluoxetine antinociceptive actions were almost similar in degree to that of Aspirin. This shows that Fluoxitine is more potent antinocipetive drug than asprin because Fluoxitine 10mg/Kg produced similar antiniceptive effects of Aspirin 100mg/Kg.

Conclusion

Within the limitations of the present studyit is concluded that Fluoxetine has antinociceptive effect in experimental animal models of pain by chemically induced Writhing test in albino rats. Fluoxetine has produced similar antinociceptive effect at doses of 10 mg/ kg when compared to Aspirin (100mg/Kg) in albino rats. Indicating Fluoxetine is more potent antinociceptive drug than Aspirin in the acetic acid induced writhing model in albino rats.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

References

1. Sona Chaturvedi, Amit chaturvedi. Post operative pain and its management. Indian Journal of Critical Care Medicine. Oct-Dec 2007; 11(4): 204-211.
2. Muralidhar Joshi. Text book of pain management. 1st ed. Hyderabad: Paras Publishers; 2005:p 4-6.
3. H.L.Sharma, K.K.Sharma. Principles of Pharmacology. New Delhi: Paras Medical Publishers; 2009: Opioid analgesics; p.94-100.
4. H.P.Rang, M.M.Dale, J.M.Ritter, J.Flower. Rang and Dale's Pharmacology 6th ed. Elseveir; 2007: Nonsteroidal anti-inflammatory drugs : p.224-260
5. Goodman & Gilman's The Pharmacological Basis of Therapeutics .12th ed.
6. DeLander GE, Hopkins CJ. Interdependence of spinal adenosinergic, serotonergic and noradrenergic systems mediating antinociception. Neuropharmacol. 1987;26:1791-4. [PubMed]
7. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 1984;7:309–338. [PubMed]
8. Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev. 2004;27:729–737. [PubMed]
9. Ren K, Dubner R. Descending modulation in persistent pain: an update. Pain 2002; 100:1–6. [PubMed]
10. Millan MJ. Descending control of pain. Prog Neurobiol 2002; 66:355–474. [PubMed]
11. Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol 2006; 80:53–83. [PubMed]
12. Mason P. Deconstructing endogenous pain modulations. J Neurophysiol 2005; 94:1659–1663. [PubMed]
13. Lu Y, Perl ER. Selective action of noradrenaline and serotonin on neurones of the spinal superficial dorsal horn in the rat. J Physiol 2007; 582(1):127–136. [PubMed]
14. Jung AC, Stagier T, Sullivan M. The efficacy of selective serotonin reuptake inhibitors for the management of chronic pain. J Gen Intern Med 1997;12:384-389. [PubMed]
15. Rafieian-Kopaei M, Sewell RDE. Newer antidepressants: Analgesia and relative monoamine reuptake inhibitory potency. J Pharm Pharmacol. 1994;46:1088.
16. Scott WAM. The relief of pain with an antidepressant in arthritis. Pract 1969;202:802-7. [PubMed]
17. Singh VP, Jain NK, Kulkarni SK. On the antinociceptive effect of fluoxetine, a selective serotonin reuptake inhibitor. Brain Res 2001;915:218-26. [PubMed]